

gene which caused amino acid to change from Thr to Met at position aa118 and researched the effects of this mutation on blood HBsAg screening test.

**Methods:** HBsAg Gene, which was amplified by PCR, was cloned and sequenced, then mutational amino acids were recovered the wild type at aa117 or/and aa118 position. By insertion of HBsAg Genes, expressing vectors were constructed and transient expressions were committed in 293T cells. Suspensions of cell culture were collected and tested by the panel which contained ten kinds of HBsAg testing kits provided by companies.

**Result:** The profile of results tested by Panel were similar between express suspension of mutation HBsAg gene and donation serum and most of kits but two failed. But the profile was significant difference compared with normal quality control serum. When mutation was recovered at 117 position, the profile of results did not change. But mutation was recovered at 118 position, the profile of results changed, and all kits can effectively test this HBsAg.

**Conclusion:** Mutation of HBsAg at 118 position which was changed from T to M led to failure tests by most of kits.

**PP-100 Induction of effective anti-HBV specific T cellular responses with dendritic cells that modified by a recombinant adenovirus vector expressing HBsAg and CTLA-4 ScFv**

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**Objectives:** To investigate the mechanism and evaluate the therapeutic potential of Ad-S-ScFv transduced DCs in HBV transgenic mice.

**Methods:** The ScFv of CTLA-4 was ligated and the recombinant Ad-S and Ad-S-ScFv were constructed. DCs were transfected with rAd (Ad-S-ScFv, Ad-S, etc), and HBV-Tg mice were randomly assigned to receive different rAd-transduced DCs twice at 3-week intervals. The HBV specific IFN- $\gamma$ <sup>+</sup> CD8<sup>+</sup> T cells proportion, the HBsAg-specific T cellular proliferation and cytotoxic activity of splenocytes were measured. The serum HBV markers and ALT levels, the histology and the expression of HBcAg, HBsAg in tissue samples of liver were also assessed. The expression and phosphorylation level for the key components of the intracellular signal pathway of MAPK and PI3K/Akt were detected.

**Results:** Compared with either DC/Ad-S or DNA immunization, DC/Ad-S-ScFv can induce much stronger type I immune responses and HBV-specific CTLs, and more significantly reduce the titer of serum HBsAg and HBV DNA, and also reduced the expression of HBcAg and HBsAg in the liver of HBV-Tg mice. The phosphorylation expression of Erk1,2 in the liver tissue of DC/Ad-S-ScFv immunized mice were significant increased, but not for the PI3K/Akt signal protein.

**Conclusion:** Ad-S-ScFv transduced DCs may be a promising candidate for a CTL-based vaccine for chronic HBV infection, and the combination with other antiviral strategy could probably acquire the complete clearance of HBV DNA.

**PP-101 Pattern of hepatitis B 'e' antigen (HBeAg) in hepatitis B surface antigen (HBsAg) confirmed positive cases**

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**Objective:** The aim of this study was to explore the pattern of HBeAg status in HBsAg true positive cases attending National Reference Laboratory of Nepal.

**Materials and Methods:** Secondary data were collected by record

review. Serum specimens confirmed for HBsAg positive cases (n=512), by neutralization method using bioKit, Spain, were subjected to semi-automatic enzyme immunoassay (EIA) GB, Taiwan for the detection of HBeAg during the period of November 2006 to February 2009. Qualitative results were calculated by cut-off formula provided in the kit insert and the produced data were analyzed.

**Results:** Of the true HBsAg positive (n=512) cases, 76.17% (n=390) were males and 23.83% (n=122) were females. Data showed that 29.49% (n=151) cases were found to be positive for HBeAg, among which 31.28% (n=122) were males and 23.77% (n=29) were females. Age wise distribution of HBeAg seropositivity showed 71.52% (n=108) cases were in the age group 21–50 years, the most active and productive age group in Nepal.

**Conclusion:** Such higher prevalence of hepatitis B virus infection in its actively multiplying phase poses a problem of greater transmission rate in the communities. As males are more mobile than females in Nepal our communities are exposed to more risk of getting hepatitis B virus infection.

Similarly, HBeAg positive mothers may give birth to babies with HBsAg positive status who ultimately become chronic carrier of hepatitis B virus.

**PP-102 The study of the CD4<sup>+</sup>CD25<sup>+</sup>treg induced by human plasmacytoid dendritic cells from chronic hepatitis B in vitro**

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**Background:** This study was undertaken to investigate whether PDCs are involved in the generation of a higher proportion of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in chronic HBV infection compared with healthy people.

**Methods:** The amount, phenotype, and function of Treg in CD4<sup>+</sup> T cells primed by PDC from 46 chronic HBV patients, 25 healthy controls, and 10 individuals with a resolved HBV infection were studied by the ways of flow cytometry, RT-PCR, ELISA and proliferation assay.

**Results:** CD4<sup>+</sup> T cells primed by PDC from chronic HBV patient were more effective than CD4<sup>+</sup> T cells primed by PDC from healthy controls and resolved HBV patients in suppressing the HBcAg-specific proliferation and the interferon production. The IL-10 and TGF- $\beta$ 1 could be also detectable in the supernatants of PDC-primed CD4<sup>+</sup> T cells. A higher percentage of Treg, defined as CD4, CD25, CD45RO, and CTLA-4-positive cells, was detected within the population of CD4<sup>+</sup> T cells primed by PDC from chronic HBV patients compared with healthy controls and individuals with a resolved HBV infection. Accordingly, CD25<sup>+</sup>Treg from PDC-primed CD4<sup>+</sup> T cells displayed a high Fox P3 messenger RNA level. Depleting CD4<sup>+</sup>CD25<sup>+</sup> Treg from CD4<sup>+</sup> T cells primed by PDC from chronic HBV patients, healthy volunteers and resolved HBV patients made PDC-primed CD4<sup>+</sup> T to lose the capability in suppressing HBV-specific T-cell.

**Conclusion:** PDCs from the patients with chronic HBV infection induce the generation of a higher proportion of CD4<sup>+</sup>CD25<sup>+</sup> Treg compared with the healthy peoples.

**PP-103 Abnormal expression of TGF- $\beta$ 1 and IGF-II associated with HBV replication in human hepatoma tissues**

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**Background:** The abnormality of many growth factor expressions involved in the formation and development of HCC, and associated with the alteration of gene methylation status. However, their molecular mechanism and expression rule remains not too clear. In the present study, we investigated the relationship

between HBV replication and the expressions of transforming growth factor (TGF)- $\beta$ 1 and insulin-like growth factor-II (IGF-II) in tissues of hepatocellular carcinoma (HCC).

**Methods:** Liver HBV-DNA was detected by *in situ* molecular hybridization technique, and the expressions of TGF- $\beta$ 1 and IGF-II were detected by the immunohistochemistry, and TGF- $\beta$ 1 mRNA and IGF-II mRNA were amplified by nested-PCR assay in HCCs and their self-control non-cancerous tissues. The relationship was investigated between TGF- $\beta$ 1 or IGF-II expression and HBV replication or their clinical pathological characteristics.

**Results:** The stronger expressions (83.3%) of TGF- $\beta$ 1 and IGF-II were found, and the incidences of TGF- $\beta$ 1 mRNA and IGF-II mRNA were 100% in HCC tissues. A significant difference was presented between in HCC tissue and in non-cancerous liver tissues ( $P < 0.01$ ). The positive rate of TGF- $\beta$ 1 in HCC was correlated to tumor differentiation, but neither to tumor size nor numbers ( $P > 0.05$ ). The levels of TGF- $\beta$ 1 and IGF-II expression were significantly associated with HBV replication with higher HBV-DNA-positive HCC (94.7%) than that in HBV-DNA-negative group.

**Conclusion:** TGF- $\beta$ 1 and IGF-II in HCC are overexpressed and associated with hepatic HBV replication and differentiation degree of HCC.

#### PP-104 Chronic hepatitis B: molecular, epidemiological and clinical features in northwest and central Russia

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**Objectives:** To reveal the HBV genotype distribution and clinical differences between different genotypes in patients with chronic hepatitis in 2 regions of Russia, to determine the structure of HBV DNA fragments responsible for resistance to treatment.

**Methods:** HBV genotyping was carried out by original PCR method. Sequencing with original primers was performed. Real time PCR was used to determine viral load in patients' blood and liver biopsies. Disease severity was evaluated by clinical-laboratory markers and morphology study.

**Results:** 325 patients with chronic hepatitis B (Mean age - 34 years. Female to male ratio - 2:1) were observed. HBV genotype was determined in 294 patients from Saint Petersburg region and 31 patients from Central part of Russia (Perm city). HBV D genotype was revealed in 96% of cases in North West and in 67% of cases in Central Russia. In the rest of cases A genotype was determined. More severe disease with necro-inflammatory infiltration and fibrosis progression was diagnosed in patients with HBV A genotype. Some patients from this group had hepatocirrhosis. 95% of patients with HBV D genotype were HBeAg-negative. HBV polymerase gene fragment sequencing from 28 patients revealed high variability of viral genome and YMDD-motif characteristic for wild type (lamivudin susceptible).

**Conclusion:** HBV genotypes A and D are circulating in North West and Central Russia. The percentage of prevalent D genotype is much higher in the North West region. Prevalence of mutant D genotype HBV enforces to include nucleoside analogues in therapeutic course of HBV chronic patients.

#### PP-105 Development of an ELISA kit for detection of HBsAg in human serum

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**Background:** Hepatitis B virus, the cause of serum hepatitis, is classified as a hepadnavirus. HBV is responsible for acute and chronic infections and may ultimately lead to cirrhosis or primary hepatocellular carcinoma. HBV have various antigens, the important of which is surface antigen or HBsAg.

The particles containing HBsAg are antigenically complex. In contrast to the other HBV antigens, HBsAg is an important diagnostic marker of an active hepatitis B infection. Emphasis of this study is to develop a direct sandwich ELISA method for detection of HBsAg in the human serum.

**Methods:** In summary, the surface of well of microplate was coated by mouse monoclonal anti-HBs with the concentration of 1 J.1g1mTl.he serum or plasma sample added to the wells of microplate after they had been saturated by BSA. Then, diluted anti-HBs-HRP (1/8000) was added to each well which was able to connect to the trapped HBsAg. The final colorimetric detection of HBsAg is performed by adding a solution of substrate of peroxidase enzyme to each well. The color intensity is directly proportional to the concentration of HBsAg in serum.

**Results:** The sensitivity and specificity of the developed method was studied on 1350 serum samples. The results indicated a 96% sensitivity and 98.8% specificity. The precision of this method was determined by the %CV for inter-assay and intra-assay.

**Conclusions:** We can develop a direct sandwich ELISA method for detection of HBsAg in the human serum with 96% sensitivity and 98.8% specificity.

#### PP-106 Analysis of lymphocyte subsets of peripheral blood in patients with acute self-limited hepatitis B

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**Objectives:** To investigate changes and significance of lymphocyte subsets (T lymphocytes, B lymphocytes, NK cells and T cell subsets) of peripheral blood in patients with acute self-limited hepatitis B (AHB) with changes.

**Methods:** By flow cytometry (FCM), immune cells of peripheral blood were compared among 23 cases of self-limited acute hepatitis B patients, 36 patients with chronic hepatitis B (CHB) and 32 healthy controls; CD4<sup>+</sup>/CD8<sup>+</sup> and ALT were monitored dynamically, meanwhile the relation between T lymphocyte subsets and ALT were explored.

**Results:** The level of CD3<sup>+</sup> T cells (75.02 $\pm$ 8.71%), CD3<sup>+</sup>CD4<sup>+</sup>T cells (43.32 $\pm$ 6.73%) and the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> (2.35 $\pm$ 0.51%) of AHB have significantly increased compared to CHB group (62.48 $\pm$ 11.33%, 33.07 $\pm$ 9.67%, 1.14 $\pm$ 0.31% respectively) and healthy group (64.00 $\pm$ 11.54%, 34.41 $\pm$ 7.53%, 1.41 $\pm$ 0.61% respectively); Dynamic monitoring of CD4<sup>+</sup>/CD8<sup>+</sup>, ALT, CD4<sup>+</sup>/CD8<sup>+</sup> had an increased trend, accompanied by lower ALT. And CD4<sup>+</sup>/CD8<sup>+</sup> had no significant relation to HBV-DNA for HBV-DNA positive AHB.

**Conclusion:** Immune status of AHB, compared to CHB and healthy controls, were significantly different and changes of T lymphocyte subsets were related to progress of disease.

#### PP-107 Up-regulation of expression of B7-H1 and its receptor PD-1 on PBMC by HBeAg

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**Objectives:** To study HBeAg does impact on molecule expression of B7-H1 and PD-1 as well as TLR2 on PBMC.

**Methods:** PBMC from different infectious status of CHB patients were stimulated by recombinant HBeAg, and expression of B7-H1, PD-1 and TLR2 on PBMC before and after stimulation as well as changes in lymphocyte subsets were quantitatively analyzed on FACS, changes in expression of PBMC surface receptor above were further observed by using blocked method of HBeAb, in order to confirm HBeAg-specific function, while analyzing growth and decline of cytokines in culture supernatant before and after stimulation.

**Results:** When PBMC from HBeAg-negative CHB patients and